Hyperbaric pressure effects on voltage-dependent Ca^{+2} channels: Relevance to HPNS

BEN AVINER[1], YEHUDIT GNATEK[1], GIDEON GRADWOHL[2], YORAM GROSSMAN[1]

[1] *Department of Physiology and Neurobiology, Faculty of Health Sciences and Zlotowski Center of Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva, Israel;*
[2] *Medical Engineering Unit, Department of Physics, The Jerusalem College of Technology, Jerusalem, Israel*

CORRESPONDING AUTHOR: Ben Aviner – *aviner@bgu.ac.il*

ABSTRACT

Known and unpublished data regarding hyperbaric pressure (HP) effects on voltage dependent-Ca^{2+} channels (VDCCs) were reviewed in an attempt to elucidate their role in the development of high-pressure neurological syndrome (HPNS). Most postulated effects from studies performed in the last two decades (*e.g.*, depressed maximal current) rely on indirect findings, derived from extracellular [Ca^{2+}] manipulation or by observing Ca^{2+}-dependent processes. More recent experiments have tried to directly measure Ca^{2+} currents under high pressure conditions, some of which are potentially challenging previous indirect findings on one hand, but support findings from work done on neuronal behavior on the other. Additional support for some of the recent findings is provided by computer simulation of pressure effects on a spinal motor neuron activity. HP effect on different types of VDCCs seems to be selective – *i.e.*, HP may suppress, facilitate or not change their activity. Thus, the specific distribution of the various types of the channels in each synaptic terminal or throughout the neuron will determine their function and will influence the neuronal network behavior under HP. Further research is needed in order to fully understand the HPNS etiology.

❖

INTRODUCTION

Mankind has conquered soil more than 376,000 km above sea level, landing humans on the Earth's moon repeatedly, yet the farthest descent accomplished with a manned submarine, the *Trieste*, in January 1960, was the sole attempt, reaching 10.9 km below the ocean surface.

Whereas many species have been adapted to life under great pressures in their search of new browses in the continuum of evolution, humans have remained quite limited in that sense. On a planet covered 70% by oceans, about 70% of which are deeper than 2-3 km (20-30 MPa), if humans are ever to explore the abyss – even with the aid of supreme technical support – our pressure susceptibility must be studied in order to remove restrictions that prevent us from entering the frontiers of the deep oceans.

Neurophysiological effects of pressure

Hyperbaric environments present many physiological challenges, especially affecting the lungs, hollow viscera and the nervous system. Under pressure, soft tissues of the body behave as a fluid and rapidly transmit any pressure applied against the surface of the body to the adjacent fluid compartments. This results in hydrostatic compression of the cerebral spinal fluid, cerebral circulation, and extracellular and intracellular fluid compartments of the mammalian CNS. Thus, practically every cell is exposed to the ambient pressure.

Common neurological problems associated with hyperbaric environments included oxygen toxicity, which is thought to occur through increased oxidative stress, as well as nitrogen narcosis (inert-gas narcosis) and high-pressure neurological syndrome (HPNS) [1,2]. Of these neurological problems, all but HPNS can be alleviated and even eliminated by controlling partial pressures of absorbed tissue gases at normal values while under pressure, leading to the notion

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that HPNS occurs due to effects of pressure *per se* [3]. HPNS signs and symptoms include vision and auditory disturbances, dizziness, nausea, reduction of cognitive functions, decreased motor coordination, sleep disorders and electroecephalogram (EEG) changes.

Although muscle performance at HP was altered [4], HPNS signs and symptoms are generally associated with signs of CNS hyperexcitability and EEG changes [5]. These affect the performance of deep sea divers exposed to pressures above 1.0 MPa [6] in a manner that risks their lives and health. At greater pressures (as in deeper diving), serious signs such as tremors, convulsions and seizures leading to death may occur [1].

An individual susceptibility to the hyperbaric environment was found in both human and animal experiment [7,8]. The pressure threshold for HPNS also seems species-dependent, with an inverse relation to the complexity of their central nervous system. Complete seizures have been seen in fish at 5-13 MPa, in reptiles at 10-13 MPa, rodents at about 9 MPa, and in primates at 6-10 MPa [9]. Tremors became apparent in humans exposed to pressures of 2.5 MPa, which progressed to myoclonus at 5 MPa [10].

It is conceivable that this constellation of signs and symptoms arises from brain malfunction that probably reflects changes in intrinsic neuronal properties and disturbances in network synaptic activity.

Molecular effects of pressure

Effects on synaptic transmission

The synapse is an interface between two cells where intercellular communication takes place, thereby enabling the formation of neuronal networks. Transmission across the chemical synapse is attained by the release of neurotransmitter molecules from the presynaptic terminal that bind to the postsynaptic membrane receptors of the target cell and produce synaptic potential. Pressure profoundly depressed synaptic transmission at all synapses examined so far, including individual synapse [11], neuromuscular junction (NMJ) [12,13], excitatory and inhibitory synapse [14,15] and in vertebrates and invertebrates [10]. A 50-70% depression of glutamatergic excitatory post-synaptic potential (EPSP) at 10 MPa was demonstrated in the crustacean neuromuscular synapses [12,15,16] and in the squid giant synapse [17], while a more modest effect of pressure was observed in cholinergic responses: nicotinic transmission in mammalian NMJ [18], muscarinic response in cervical sympathetic ganglion [19], and in cholinergic synapses in mollusks [11]. Pressure has also been shown to reduce population field EPSP (pEPSP) in rat hippocampal [20,21] and dentate gyrus [22,23] brain slices, and in guinea pig cerebellar Purkinje cells [24]. The latter study also suggested for the first time that this reduction could be attributed to a specific Ca^{2+} channel-dependent component of the pEPSP *(N-type)*.

The obvious question is what stage of synaptic transmission is the pressure-sensitive one? Several lines of evidence suggest that pressure predominantly affects presynaptic mechanisms. First, since transmitter release has common properties across various synapses whereas post-synaptic responses differ considerably, the given uniformity of the pressure effect at all synapses suggests a presynaptic site. Second, several changes induced by pressure at synapses are of properties associated with events at the presynaptic terminal:

a. HP markedly and reversibly depressed spontaneous miniature end-plate potentials frequency in the frog NMJ, without a noticeable change in its mean amplitude (probably due to its dual effect of reducing the amplitude and lengthening the decay time of the miniature end-plate currents; thus the receptor's charge transfer remains the same) [25];
b. HP increased facilitation and tetanic potentiation [15].
c. Evidence from synaptosomes (sealed vesicles from broken nerve terminals, containing Ca^{+2} channels and the synaptic release apparatus) showed slowed release and in some cases a moderate reduction in the maximal release [26], with the exception of the aspartate synapse [27].
d. When the presynaptic mechanisms were bypassed by direct application of the neurotransmitter ACh, pressure had no effect on the response in helix neurons [11].

However, it is important to note that there are changes in the kinetics of excitatory post synaptic potentials (EPSPs) and excitatory post-synaptic currents (EPSCs) in most synapses, as well as pressure modulation of specific ligand-gated ion-channels, that will contribute to the depression mechanisms through post-synaptic effect [10].

Overall, most evidences point towards a presynaptic mechanism for pressure depression of synaptic transmission, and many of the effects can be explained by depression of Ca^{2+} influx into the presynaptic terminal, through voltage-dependent Ca^{2+} channels (VDCCs), which is the trigger for the subsequent steps of synaptic transmission. Furthermore, low $[Ca^{2+}]_o$ mimics the effects of HP [13,28], leading together to the notion that the VDCCs are indeed involved in this depression.

Effects on voltage-dependent ion channels

Ion channels are transmembranal proteins, the function of which is associated with conformational changes. The specific ion (negative or positive) influx or efflux across the membrane (depending on the ion electrochemical gradient) determines its effect on the membrane potential. Voltage-dependent channels are mainly modulated by the membrane potential, usually activated by membrane depolarization, and deactivated when the potential recovers to resting level. Many of the channels also exhibit voltage-dependent inactivation that occurs during maintenance of membrane depolarization. Voltage-dependent Na^+ and K^+ channels are responsible for the generation and conduction of action potential (AP) along neuronal axons and muscle fibers, and evidence has accumulated to show that AP duration is lengthened at HP [29-32].

Pressure effect on voltage-gated Na^+ channels varies between relatively moderate [32,33] to significant [34] reduction of action potential Na^+ current amplitude and slowed its activation and inactivation. When voltage-dependent K^+ channels were examined at HP, most studies have shown K^+ currents to be enhanced [35-38], while others have suggested their depression [36,39]. In the following paragraphs we will discuss in detail HP effect on VDCCs.

Voltage dependent Ca^{2+} channels

VDCCs mediate Ca^{2+} influx in response to membrane depolarization. This transient Ca^2+ influx serves as the second messenger of electrical signaling, initiating intracellular events such as neurotransmitter release from presynaptic terminals, neuronal excitability, excitation-contraction coupling in cardiac muscles, hormone secretion, ciliary movement and gene expression.

General structure

VDCCs are members of a gene super family of transmembranal ion channel proteins that includes voltage-gated K^+ and Na^+ channels [40,41]. Various VDCC types exist, composed of four or five distinct subunits (α_1, $\alpha_2\delta$, β, γ) that are encoded by multiple genes [42]. Their general organization is illustrated in Figure 1 (*see Page 248)*.

α_1 subunit: The largest subunit (190-250 kDa) that holds the ion conduction pore, the voltage sensor, the channel gating area and most of the known sites of channel regulation by second messengers, drugs, and toxins [43]. Like the α subunit of the sodium channel, it is organized in four homologous domains (I-IV), each consisting of six transmembranal helices (S1-S6) and a P-loop between S5 and S6 that together form the channel's pore. This loop determines the channel ion conductance and selectivity. Upon membrane depolarization the positively charged S4 segment, which functions as the voltage sensor for activation, moves outward and rotates, thus initiating a conformational change that opens the pore.

β subunit: An intracellular protein, 52-78 kDa, that can interact with and modulate α1 subunit [42, 44].

$\alpha_2\delta$ subunits: Transmembranal disulfide-linked proteins (175 kDa). The δ section is anchored to the membrane, while the α_2 subunit is entirely extracellular [44].

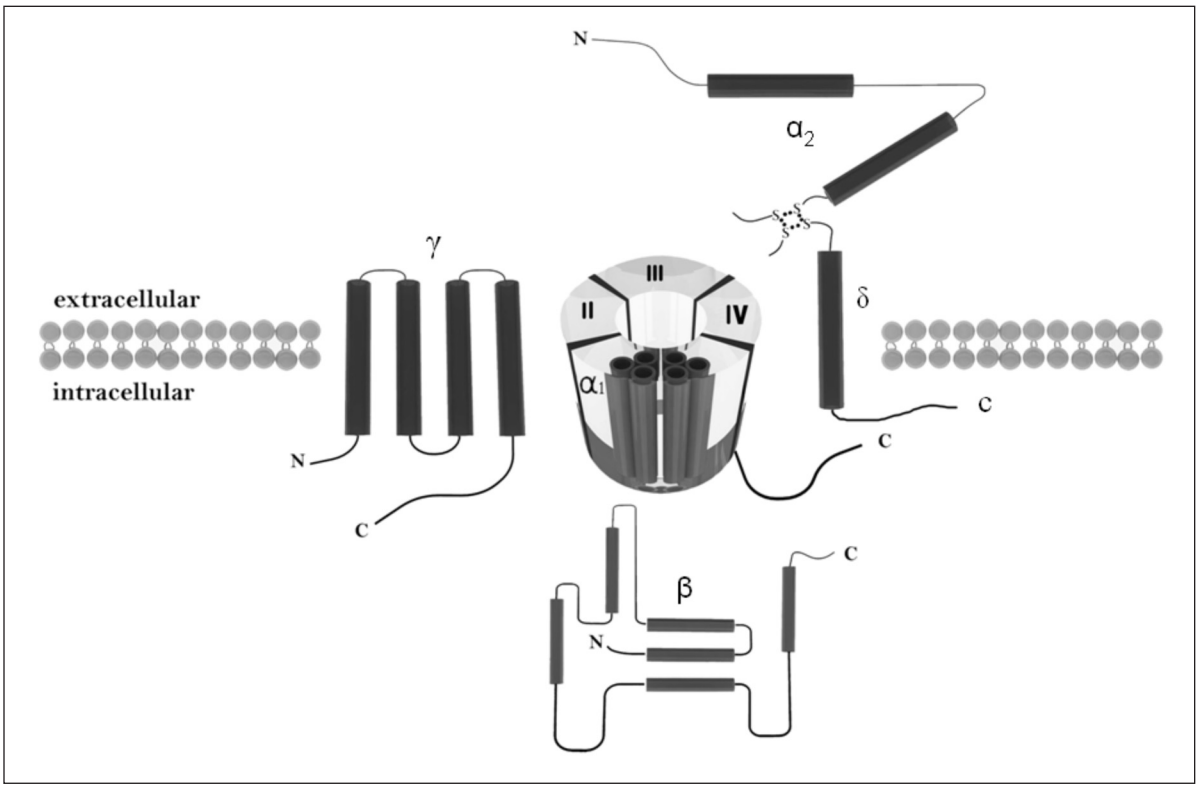
γ subunit: Composed of four transmembranal helices (33 kDa). No evidence was available as to the exact role of this subunit in trafficking or regulating of the channel complex for most channel types. However, a recent study has shown that it does have a role in modulating the $Ca_v1.1$ channel [45] *[see the following subheads: "Nomenclature" (below) and "Physiological and pharmacological properties" (Page 248)]*.

Ten α_1, four β, four $\alpha_2\delta$ and eight γ subunits isoforms are known to date, attesting to the wide diversity of the VDCCs and their functional properties. Although these supporting subunits modulate the properties of the channel complex, the pharmacological and physiological diversity of Ca^{2+} channels arises primarily from the existence of multiple α_1 subunits [46].

Nomenclature

In 2000, a systematic nomenclature was adopted [43], based on the α_1 various isoforms. Ca^{2+} channels were named using the chemical symbol of the principal permeating ion (Ca) with the principal physiological

FIGURE 1 – ***Spatial organization of the subunits constructing the VDCC***



regulator (voltage) indicated as a subscript (Ca_v). The numerical identifier relates to the gene subfamily of the α_1 subunit (1 to 3 at present) and the order of discovery of the α_1 subunit within that subfamily (1 through *n*). These three subfamilies correspond with the distinct classes of Ca^{2+} currents *(see below)*, previously used as the classifier parameter. The Ca_v1 subfamily ($Ca_v1.1$-$Ca_v1.4$) includes the *L-type* Ca^{2+} currents. The Ca_v2 subfamily ($Ca_v2.1$- $Ca_v2.3$) includes the *P/Q-type, N-type* and *R-type* Ca^{2+} currents. The Ca_v3 subfamily ($Ca_v3.1$- $Ca_v3.3$) includes the *T-type* Ca^{2+} currents *(see Table 1, facing page)*.

Physiological and pharmacological properties

The different Ca^{2+} currents were defined by physiological and pharmacological properties [47-49] *(Table 1)*.

L-type currents (Ca_v1) require high voltage for activation (HVA), have high single-channel conductance and inactivate slowly during depolarization. They are the main Ca^{2+} currents recorded in muscle and endocrine cells, where they initiate contraction and secretion [50]. *L-type* currents can also be found in cardiac muscle and neuronal dendrites and soma [51], where they are involved in regulation of gene expression and in integration of synaptic input [47]. This family is blocked by organic antagonists, including dihydropyridine (DHP) and is regulated primarily by protein phosphorylation through a second messenger-activated kinase pathway [42].

N-type, P/Q-type, and *R-type* currents ($Ca_v2.1$, $Ca_v2.2$ and $Ca_v2.3$ respectively) are HVA channels, insensitive to organic *L-type* channel blockers but are blocked by specific polypeptide toxins from snail and spider venoms [49]. This family is predominantly expressed in the neurons, where they initiate neurotransmission and mediate Ca^{2+} entry into cell bodies and dendrites. However they can also be found in the heart, pituitary, pancreas and testes [50]. Ca_v2 channels are regulated by direct binding of soluble NSF attachment receptor (SNARE) proteins and GTP binding proteins, and that primary mode of regulation is itself regulated by protein phosphorylation pathways [42].

T-type currents (Ca_v3) require low voltage for activation (LVA), inactivate rapidly, deactivate slowly, have small single-channel conductance [52] and are resistant to Ca^{2+} channel antagonists. They are expressed in a variety of cell types, including neuronal cell bodies and dendrites, where they are involved in shaping the AP and controlling pattern of repetitive firing [50]. The molecular mechanisms of the Ca_v3 channel regulation are currently unknown.

TABLE 1 – *Ca^{2+} channel types*

CHANNEL	CURRENT	LOCALIZATION	SPECIFIC ANTAGONIST	CELLULAR FUNCTION
$Ca_V1.1$	L	Skeletal muscle; transverse tubules	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; Excitation-coupled-Ca^{2+} entry *
$Ca_V1.2$	L	cardiac myocytes; smooth muscle myocytes; endocrine cells; neuronal cell bodies; proximal dendrites	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; hormone release; regulation of transcription; synaptic integration
$Ca_V1.3$	L	endocrine cells; neuronal cell bodies and dendrites; cardiac atrial myocytes and pacemaker cells; cochlear hair cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Hormone release; regulation of transcription; synaptic regulation; cardiac pacemaking; hearing; neurotransmitter release from sensory cells
$Ca_V1.4$	L	retinal rod and bipolar cells; spinal cord; adrenal gland; mast cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Neurotransmitter release from photoreceptors
$Ca_V2.1$	P/Q	nerve terminals and dendrites; neuroendocrine cells	ω – Agatoxin IVA	Neurotransmitter release; dendritic Ca^{2+} transients; hormone release
$Ca_V2.2$	N	nerve terminals and dendrites; neuroendocrine cells	ω – Conotoxin GVIA	Neurotransmitter release; dendritic Ca^{2+} transients; hormone release
$Ca_V2.3$	R	neuronal cell bodies and dendrites	SNX-482	Repetitive firing; dendritic Ca^{2+} transients
$Ca_V3.1$	T	neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
$Ca_V3.2$	T	neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
$Ca_V3.3$	T	neuronal cell bodies and dendrites	None	Pacemaking; repetitive firing

TABLE 1: Subunit composition and function of Ca^{2+} channel types, modified from [50]; * [53] added.

Pressure effects on voltage-dependent Ca^{2+} channels

Synaptic release is a multistep mechanism. The first crucial stage is Ca^{2+} influx into the presynaptic terminal and elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) following membrane depolarization by the invading AP. Increased $[Ca^{2+}]_i$ leads to fusion of docked vesicles with the terminal plasma membrane, ending in evoked neurotransmitter release. As noted above, most evidence support presynaptic mechanisms as the underling cause of pressure depression of synaptic transmission. Decreased Ca^{2+} influx into the presynaptic terminal appears to be a good explanation for many of these effects.

Indirect evidence

Most available data on VDCCs under pressure are indirect evidence, acquired by manipulating extracellular Ca^{2+} concentrations ($[Ca^{2+}]_o$) or by observing Ca^{2+}-dependent functions. Such a function was studied in the *Paramecium*, where the brief reversal of swimming direction is Ca^{2+}-dependent. Normally the reversal occurs when the protozoan encounters the container wall. Under 10 MPa hydrostatic pressure this brief reversal of swimming direction was inhibited [54]. Furthermore, spontaneous reversals induced by Ba^{2+} were blocked during pressurization, suggesting that pressure decreases Ca^{2+} influx through the *Paramecium's* unclassified VDCC. This was supported by studies comparing pressure effects with the responses under different $[Ca^{2+}]_o$.

A theoretical model for transmitter release in crustaceans has been developed by Parnas *et al*. (1982) [55] in which the release process is divided into three main steps: 1) Ca^{2+} entry; 2) neurotransmitter release; and 3) removal of intracellular Ca^{2+}, each step with its specific characterizations. Studies on crustacean neuromuscular synapses examined the relationship between $[Ca^{2+}]_o$, EPSC amplitude and facilitation using this model [13,15,56]. The analysis indicated that pressure was acting to reduce Ca^{2+} influx, rather than to affect intracellular removal of Ca^{2+} or the release process. In addition, decreased $[Ca^{2+}]_o$ mimicked the pressure effect on EPSC's amplitude, while increasing $[Ca^{2+}]_o$ above normal levels antagonized its effect. Furthermore, application of various Ca^{2+} channel blockers aggravated the depressant effect of pressure on crustacean EPSCs, supporting the notion that HP depresses synaptic response by impeding Ca^{2+} influx [57]. Similar effect of $[Ca^{2+}]_o$ was reported for CNS single pEPSPs in the hippocampal dentate gyrus [58]. In contrast, HP had little effect on the curve relating $[Ca^{2+}]_o$ and single spinal cord monosynaptic reflex response (a measurement of dorsal root compound AP) in newborn rats and did not change its saturation level [59]. The slow after-hyperpolarization (sAHP) amplitude of the AP was reduced by HP in rat CA1 [39], a reduction which could be explained by a depression of the SK potassium channel, responsible for the sAHP. But this channel is activated by the rise of $[Ca^{2+}]_i$ during each AP, potentially pointing to a reduction in Ca^{2+} influx through VDCCs.

Previous studies have demonstrated colocalization of different VDCCs in single motor nerve terminals of frog [60], mouse [61] and CNS terminals [62] as well as the presence of various VDCCs involved in transmission in the CNS [63,64]. This non-homogeneous expression of VDCCs is probably manifested in different responses to HP among various species and different synapses in a given species, according to the channels sensitivity to pressure. Indeed, at crustacean neuromuscular synapses, the Ca^{2+} channel involved in transmission resembles the vertebrate *N-type* channel and, as mentioned above, this transmission is depressed under pressure conditions, probably due to reduction of Ca^{2+} influx through the VDCC [57]. A study by Etzion and Grossman (2000) [24] in cerebellar Purkinje cells support these findings. When non-selective reduction in Ca^{2+} influx was employed (Cd^{2+} application or low $[Ca^{2+}]_o$), partial synaptic depression occurred, and pressure substantially added to this depression. However, following a similar partial block by a selective *N-type* Ca^{2+} channel blocker (CTX), pressure had almost no additional effect, strengthening the hypothesis that pressure blocks mainly the *N-type* channel.

HP slightly increased the apparent synaptic delay, partially due to a decrease in axonal conduction velocity [35]. However, simultaneous measurement of the nerve terminal current and EPSCs uncovered a pressure effect on synaptic delay *per se* [56]. Under normal conditions $[Ca^{2+}]_o$ does not affect synaptic delay. Yet, at 10.1 MPa, decreasing $[Ca^{2+}]_o$ increased synaptic delay. The apparent activation volume of the pressure sensitive reaction is reminiscent of the pressure effect on ionic channels, but also of the exocytosis mechanism itself, which seems to be depressed by HP [25]. Endocytotic membrane retrieval, another presynaptic Ca^{2+} influx-dependent

process [65, 66], is also inhibited by HP [67], further supporting the hypothesis that impeded Ca^{2+} flux has a substantial role in the synaptic transmission malfunction at HP.

In the context of indirect studies, it is important to note that pressure might interfere with Ca^{2+} action within the terminal rather than decreasing flux (*e.g.*, vesicle fusion and exocytosis). Furthermore, reducing $[Ca^{2+}]_o$ can also have postsynaptic effects – *e.g.*, on the glutamate receptor [26].

Direct evidence

Although only a few works performed direct measurements of Ca^{2+} currents and uptake, the available studies reinforce the findings mentioned above.

Ca^{2+} uptake by synaptosomes

Early measurements of voltage-dependent radiolabeled Ca^{2+} uptake into brain synaptosomes, revealed that HP depresses its uptake [68], supporting the concept of decreased Ca^{2+} influx due to HP. To further test this concept, Gilman *et al*. (1991) [69] used artificially added Ca^{2+} ionophore (A23187) to bypass Ca^{2+} channels and examined pressure effects on Ca^{2+} influx through the ionophore and consequent radiolabled GABA release. HP slightly increased the Ca^{2+} influx, but depressed the release. These results indicate that, although pressure probably diminishes Ca^{2+} influx through VDCCs, it also affects processes subsequent to Ca^{2+} entry, such as vesicle fusion [25,70] and endocytotic membrane retrieval [67].

Ca^{2+} current measurements

In bovine chromaffin cells, direct measurements of Ca^{2+} currents did not show any significant alteration (only a very small increase in some experiments) after pressurization to 40 MPa [70]. The channel in these cells has a similar kinetic behavior to the *L*- and *P/Q-type* channels in other neurons, suggesting that, unlike the *N-type* channel, these channels are resistant to pressurization. It has also been reported that similar resistance to pressure is obtained for *P-type* Ca^{2+} action potentials in guinea pig cerebellar Purkinje cells [71]. On the other hand, Ca^{2+} current measured in a rat skeletal *L-type* channel following decompression from HP (20 MPa) was reported to be affected by the treatment, with reduced peak amplitude, prolonged time-to-peak and slower current decay [72].

The effect of pressure on two types of colocalized Ca^{2+} currents was first tested in the frog motor nerve [26,34]. In addition to the action potential Na^+ current of the axons, blocking K^+ channels using tetraethylammonium (TEA) revealed a slower Ca^{2+}-dependent current comprised of fast (I_{CaF}) and slow (I_{CaS}) components [73] that reflect the Ca^{2+} inward current at the terminals. Both phases were blocked by Cd^{2+} and ω-conotoxin (*N*- and *L-type* blockers), but only I_{CaS} was diminished by nifedipine and nitrendipine (*L-type* blockers). Pressurization to 6.9 MPa suppressed I_{CaF} by about 87% , whereas I_{CaS} was much less sensitive to pressure (29% reduction) and was partially restored by increased $[Ca^{2+}]_o$ [34]. These results could theoretically be derived from a reduction in nerve terminal depolarization by the invading AP. To verify that the decline in current is a direct effect of pressure on the VDCCs, the terminal was depolarized directly via the electrode. Similar results were obtained (Aviner *et al.*, unpublished data). These results further strengthen the concept that pressure exerts a differential effect on various types of VDCCs at the nerve terminal.

Studies in oocytes

A widely utilized expression system of ion channels is the *Xenopus* oocyte, which has the ability to synthesize exogenous protein when injected with foreign mRNA [74]. In this preparation, along with the possibility to directly measure the channel currents, one can express a certain channel type from a chosen species, down to the specific isoforms composing it. Consequently, the responses are an exclusive result of the overexpressed channel almost without interfering "noise." Furthermore, this setup enables a more detailed and systematic study of the channel's kinetics in addition to its maximal current.

In preliminary studies by Aviner *et al.* [75,76], a rabbit's $Ca_v3.2$ *T-type* Ca^{2+} channel (TTCC) and $Ca_v1.2$ *L-type* Ca^{2+} channel (LTCC) were expressed separately in oocytes. HP significantly reduced the maximal current of the $Ca_v3.2$ at relatively low pressures (1.0 MPa), suggesting high sensitivity to HP. Surprisingly, HP (5.0 MPa) almost doubled the maximal currents generated by the $Ca_v1.2$. This finding may be in contrast with previous works reporting the LTCCs to be quite resistant to pressure application in frog *(Rana pipiens)* NMJ [34], in bovine adrenal chromaffin cells [70] and guinea pig Purkinje cells [57]. However, a possible explanation may be derived from the variety of VDCCs and the difficulty in their identification in each preparation. Furthermore,

FIGURE 2 – ***Simulation of motoneuron '38' spike boosting by pressure exposure***

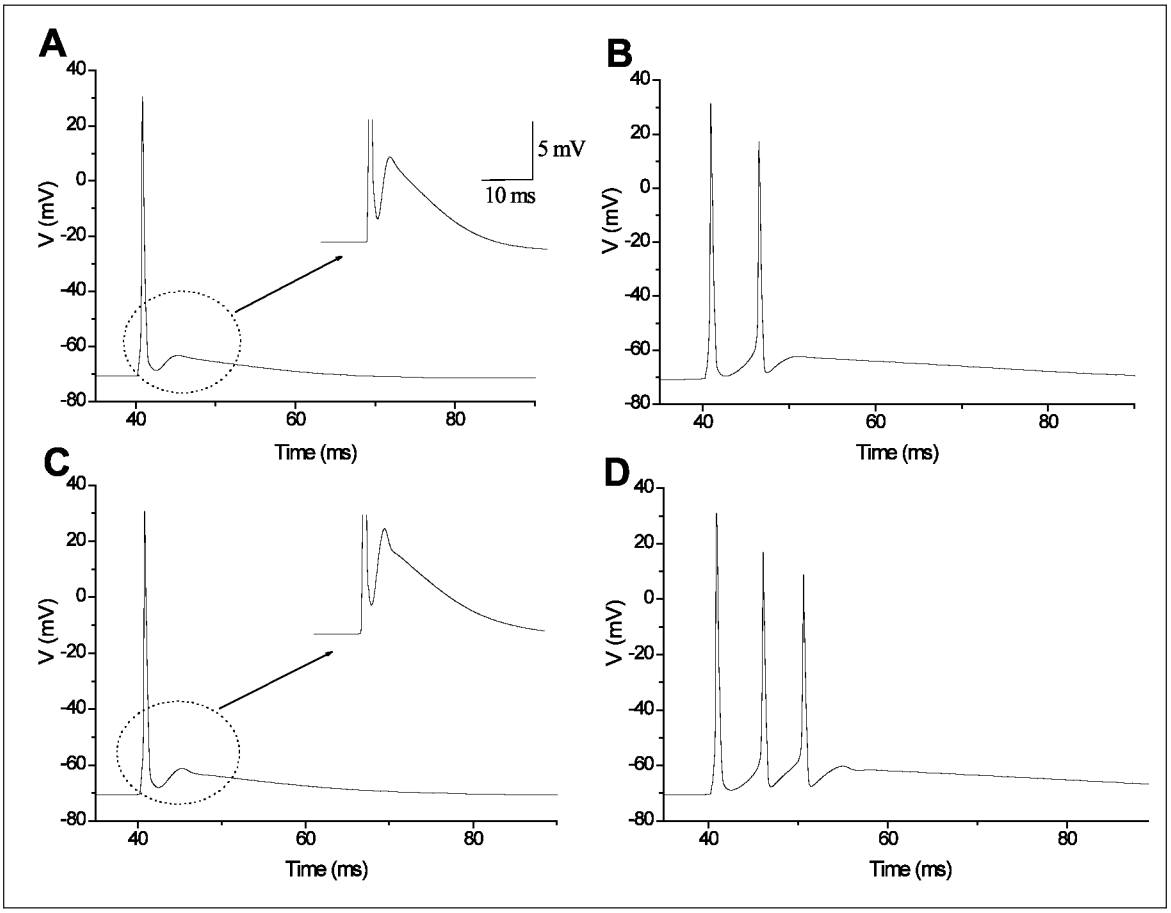


FIGURE 2: Details of the model are described in [81]. Membrane potential is shown at the soma. Na^+ and K^+ channels are incorporated in the initial segment-soma, and dendrites. LTCCs are located at the proximal dendrites 0 – 400 μm from the soma and are distributed by an exponential decay function. The included conductances (g_{NMDA} , g_{AMPA} and g_{LTCC}) of the model reflect the macroscopic conductances, since single-channel conductance is generally believed to be unaltered by HP [84-86].
A – control, action potential is evoked by a single AMPA/ NMDA EPSP.
B – pressure-induced 30% increase of g_{NMDA} and 50% longer τ decay, while g_{AMPA} was decreased by 30%.
C – pressure-induced 100% increase of dendritic g_{LTCC} alone.
D – combining both pressure effects on NMDA/AMPA and LTCC (B and C). See text for results.

more recent studies have shown that approximately half of Ca^{2+} currents in bovine chromaffin cells are mediated by $Ca_v2.1$ channels (which seems pressure-resistant, as mentioned above), and only 15-20% by $Ca_v1.2$ [77-80], which may explain the slight increase of current at HP. These results demonstrate again, on a molecular level, that HP has differential effects on various VDCCs. HP did not affect the inactivation of both $Ca_v1.2$ and $Ca_v3.2$ channels, supporting the contemporary concept of different activation and inactivation mechanisms of voltage-gated ionic channels.

Computer simulations

The possibility that the increased current in specific LTCC ($Ca_v1.2$) may explain the previously observed boosting effect of HP on depressed synaptic potential in generating population spikes in CNS neurons [20,23] is quite intriguing *(see Figure 3, facing page)*.

In order to examine this hypothesis we used a computer model simulation of "realistic" spinal motor neuron utilizing NEURON software, which was developed in our laboratory [81]. We studied the effect of HP-induced increase of NMDA receptor activity at the synaptic input [82,83] and/or increased $Ca_v1.2$

FIGURE 3 – ***Postulated HP effects, based on neuronal VDCCs distribution***

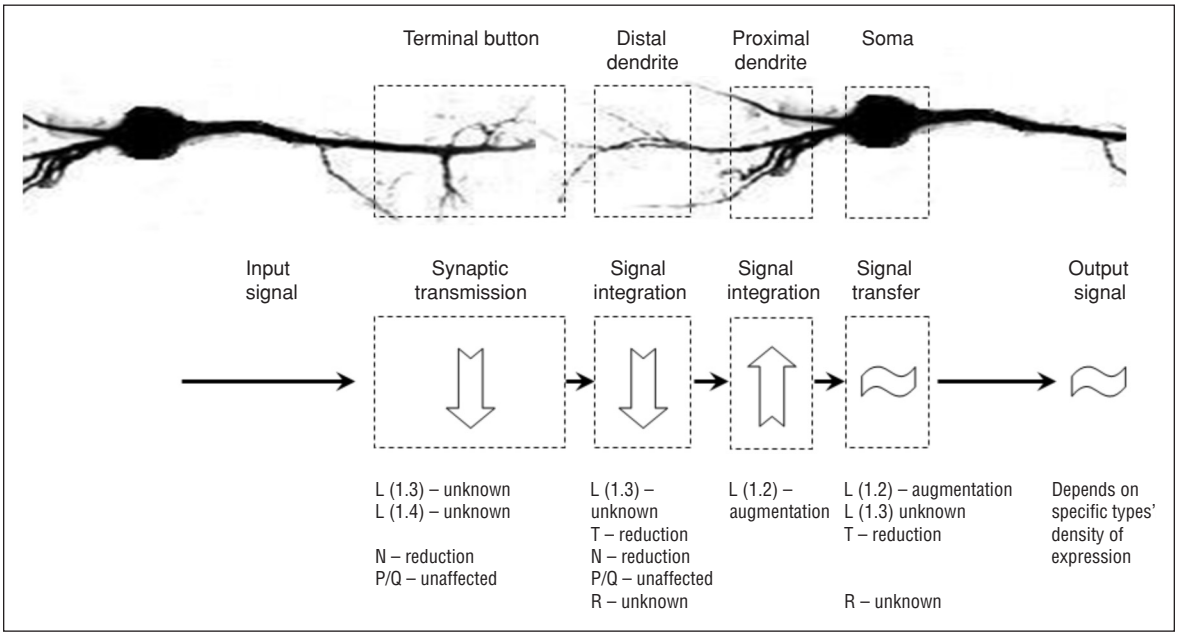


FIGURE 3 – ***Top***, schematic representation of two adjacent CNS neurons; dashed squares point to sections of the neuron in which VDCC types are known to be expressed.
Center, a flow chart describing anticipated function of signal transfer for each section.
Bottom, VDCC distribution and known HP effects on VDCC types are indicated.

activity embedded at the neuronal dendrites on the intracellularly "recorded" firing pattern of the motor-neuron in response to a single glutamatergic EPSP *(see Figure 2, facing page)*.

Under the model morphological and physiological "realistic" conditions, the enhanced glutamatergic NMDA synaptic potential, concomitantly with moderate reduction in AMPA synaptic potential, increased the number of evoked spikes *(Figure 2B)*. In contrast, increased g_{LTCC} alone, did not contribute to the number of evoked spikes *(Figure 2C)*, although the "hump" following the first action potential was enhanced due to the increase of g_{LTCC} *(inset of Fig 2C)* relative to the "hump" of the control conditions *(inset of Figure 2A)*. However, the combination of changes in both synaptic input and LTCC (B+C) increased the number of spikes to an even greater extent *(Figure 2D)*.

It is worth noticing that the gLTCC, which is partially responsible for the "hump," in Figure 2C, is activated by normal, relatively short-time g_{AMPA} and g_{NMDA}, while in Figure 2D it is activated by much greater – and especially longer – EPSP that optimize its response.

We therefore suggest that specific LTCC ($Ca_v1.2$) may boost glutamatergic EPSPs under pressure conditions.

DISCUSSION

From the available data, it is clear that pressure effects on VDCCs are selective and depend on their specific family and, possibly, sub-family. It appears that one of the more pressure-susceptible Ca^{2+} channels is the *N-type* channel, shown to be depressed under pressure [24, 34, 57]. This channel is known to be expressed in nerve terminals *(see Table 1, Page 249)*, suggesting its participation in pressure effect on synaptic depression. Nevertheless, the identification of the *N-type* channel in these studies was either by its similarity to known *N-type* channel characteristics or by pharmacological means. More direct measurements are required to establish these findings. Another channel expressed in nerve terminals is the *P/Q-type* channel, which, as mentioned above, was associated with pressure resistance in guinea pig cerebellar Purkinje cells [70,71,80]. Hence, it is conceivable that synapses in which transmission involves predominantly *P-type* channels will be much less sensitive to pressure than those involving *N-type* channels.

TTCC ($Ca_v3.2$), that presented high sensitivity to pressure [76], is found mainly in neuronal soma and dendrites, and is known to be involved especially in generating bursting behavior and rhythmic activity in pacemaker neurons [87]. Accordingly, the current reduction of this channel is expected to slow and impair the neuronal "clock" functions. HP depression of the $Ca_v3.2$ seems to be maximal at a pressure of 1.0 MPa, at which professional divers begin to experience mild HPNS. This may indicate the channel's involvement in this state of HPNS. We may speculate that the contribution of such a channel will depend on its distribution in the brain regions. For example, TTCCs are expressed at the reticular thalamic nucleus, hence disturbances of its neuronal activity could lead to changes in EEG. This indeed was demonstrated by Rostain *et al*. (1997) [88] in human divers. The thalamus is also responsible for sleep, awareness and activity periods. Therefore, interference with its performance could lead to sleep disorders on one hand, or drowsiness on the other. TTCCs are also expressed in the striatum, which has a role in executive functions, movement planning and modulation, as well as transmitting sensory inputs to the cortex. Disruption of their activity could lead, respectively, to reduced cognitive performance, impaired coordination, and vision and auditory disturbances – which, in fact, are all part of HPNS.

As mentioned above, pressure effects on the LTCCs are contradicting [34,70]. However, the $Ca_v1.2$ channel, which was augmented at HP [75], is present in the cell bodies and proximal dendrites of neurons in the dentate gyrus and hippocampus [51] *(see Table 1)*. Based on the known localization of the channel and our computer simulation, we suggest that pressure-potentiated *L-type* currents in the proximal dendrite may boost pressure-depressed subthreshold synaptic potentials to generate action potentials *(see Figure 3)*, as in fact observed in hippocampal brain slices [20,23]. Such increase in dendritic excitability could contribute to the generation of the network hyperexcitability in HPNS, by a non-synaptic mechanism.

This is a good example for another way through which pressure-selective effects on VDCC might impact neuronal networks, other than synaptic transmission.

Although analysis of synaptic release in crustaceans indicated that pressure acts to reduce Ca^{2+} influx, rather than to affect intracellular removal of Ca^{2+} or the release process [13,15,56], there is evidence for pressure depression of other presynaptic mechanisms, mainly exocytosis [70]. However, it may not play a major role in fast transmission but rather in slow secretion of neuromodulators and neurohormones.

Most evidence linking $[Ca^{2+}]_o$ and Ca^{2+} influx to the effect of HP are in single or twin responses [13,15, 56-58]. When frequency responses of different CNS synapses were examined, changing $[Ca^{2+}]_o$ did not always align with the effect of pressure and occasionally had an opposite effect [22, 89]. These studies indicate that hyperbaric pressure probably interferes with additional mechanisms of release such as exocytosis [70].

The complexity of the CNS function, the variety of VDCCs and the selective effect of pressure makes it even more challenging to point to the potential role of VDCCs in HPNS. Systematic and detailed study of the different VDCCs, in parallel to other possible pressure-affected molecules and mechanisms, will shed more light and increase our understanding of the underling processes of HPNS. This will certainly increase our ability to explore the abyss of the oceans and exploit its resources in the future.

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